

were in the following order: PBS > SUPARTZ® > 1 injection of Gel-200 ≥ 2 injections of Gel-200. Efficacy of Gel-200 for suppression of cartilage degeneration was also demonstrated by a reduction of the increase in chondroitin 6-sulfate (6S) in the synovial fluid. In addition, Gel-200 appeared to improve the symptoms of synovitis, as judged from the reduction in increase of synovial fluid, protein and chondroitin 4-sulfate (4S) contents. Overall, since cartilage degeneration is milder when synovitis is not severe, these changes induced by Gel-200 may interact beneficially to relieve the progression of pathological changes. Histopathological findings of articular cartilage supported the morphological assessment. In the histopathological examination of synovium, cuboidal/stratified synovial epithelium, subepithelial cellular infiltration, subepithelial fibrosis/edema, subepithelial hemorrhage and subepithelial calcium deposition were observed in all the experimental groups. These changes were less severe in Gel-200 groups compared to those in the control group.

Conclusions: These data show that in a rabbit ACL transection model of OA, a single intra-articular injection of Gel-200 was superior to five injections of SUPARTZ® in slowing cartilage degeneration. It is considered that this superiority of Gel-200 is attributed to its highly cross-linked structure.

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THE IN VIVO ACTIVATION OF PPAR_γ BY THE LIGAND PIOGLITAZONE REDUCES THE DEVELOPMENT OF CARTILAGE LESIONS AND SYNTHESIS OF CATABOLIC FACTORS IN AN OSTEOARTHRITIS DOG MODEL

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Purpose: The peroxisome proliferator-activated receptor (PPAR)_γ is a known modulator of a number of inflammatory pathways. Emerging evidence indicates that PPAR_γ may have protective effects on structural changes in osteoarthritis (OA). In this study we evaluated the in vivo therapeutic effect of a PPAR_γ agonist, pioglitazone, on the development of structural lesions in a dog anterior cruciate ligament (ACL) model of OA and explored the effect of the drug on the major synthetic pathways involved in the disease process.

Methods: OA was surgically induced in 24 dogs. The OA dogs were randomly divided into 3 groups (n=8 per group) and treated orally with either placebo, 15 mg/kg/day of pioglitazone, or 30 mg/kg/day of pioglitazone. The treatment began the day after surgery and continued for a period of 8 weeks. The severity of cartilage lesions was scored macroscopically. Cartilage specimens from femoral condyles and tibial plateaus were processed for histologic evaluation. Both cartilage and synovial membrane were processed for quantitative RT-PCR and immunohistochemistry. Specific probes and antibodies were used to study iNOS, MMP-1 and ADAMTS-5.

Results: Pioglitazone treatment reduced the development of cartilage lesions in a dose-dependent manner. There was a reduction in the lesion scores, and statistical significance was reached for the medial condyle; p<0.04 and 0.03 respectively for 15 and 30 mg/kg/day of pioglitazone. This decrease correlated with the cartilage histologic scores. Pioglitazone also significantly reduced the production of OA key mediators, iNOS, MMP-1 and ADAMTS-5 in cartilage.

Conclusions: This study demonstrated the efficacy of pioglitazone, a PPAR_γ agonist, at reducing articular lesions in a dog model of OA. These results provide a new possibility for therapeutic intervention in OA, in which in vivo activation of PPAR_γ inhibits major cartilage catabolic factors responsible for articular tissue degradation.

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AUTOLOGOUS OSTEOCHONDRAL GRAFTS IN THE TREATMENT OF FOCAL CHONDRAL DEFECTS OF THE FEMORAL HEAD. AN EXPERIMENTAL STUDY IN RABBITS

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Purpose: To investigate and compare the characteristics of the reconstructed articular surface microscopically and histologically after a time period of 6 weeks following the treatment of a focal defect of the right femoral head with subchondral drilling and autologous osteochondral transplantation in rabbits

Methods: A 2,5 mm diameter and 3 mm depth iatrogenic osteochondral defect in the anterolateral weight bearing area of the right femoral head was created in 12 rabbits. In a group of 6 rabbits the lesion was treated with autologous osteochondral transplantation. The donor site for the transplant was the lateral condyle of the ipsilateral knee joint. The other group of 6 rabbits was treated with subchondral drilling. Both groups were sacrificed after a time period of 6 weeks and specimens were evaluated histologically under the classification system of the ICRS. For statistical analysis we used the Mann - Wittney test

Results: According to the ICRS score statistical significance was found for all variables between the 2 groups (subchondral drilling 6 weeks vs autologous osteochondral transplantation 6 weeks): articular surface (p=0,049), matrix (p=0,003), cell distribution (p<0,0005), subchondral bone (p=0,010), cartilage mineralization (p=0,0) except cell population viability.

Conclusions: In cases of focal osteochondral defect of the femoral head in rabbits, reconstruction of the articular surface through autologous osteochondral graft transplantation gives superior macroscopical and histological results in comparison to subchondral drilling

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EFFECTS OF THE INTERLEUKIN-1 RECEPTOR ANTAGONIST ANAKINRA, ON PAIN AND GROSS PATHOLOGY IN THE MONOIODOACETATE MODEL OF OSTEOARTHRITIS

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Purpose: Interleukin-1 (IL-1) is thought to play a role in both the joint destruction seen in osteoarthritis (OA) and the pain that develops with the disease. The monoiodoacetate (MIA) model of osteoarthritis in the rat has been used as a model of OA pain and additionally, displays gross pathology and histologic changes that resemble some components of OA pathology. This study therefore uses the MIA model to examine the effect of the IL-1 receptor antagonist (IL-1RA) Anakinra on cartilage degradation and joint pain.

Methods: 20 male Lewis rats of 7 weeks of age were randomized into 2 experimental groups of n=10. The rats were then implanted with Alzet osmotic pumps (model 2004) containing either vehicle (PBS) or IL-1RA at a dose of 90mg/kg per day. Three days after osmotic pump implantation the right knees of each rat were injected with 0.3mg of MIA in 50ul of saline and the left knees with 50ul of saline. 6 rats from each group were then measured for pain on days 1, 2, 3, 8, 10 and 14 post MIA injection by incapacitance testing. This measures the difference in paw weight bearing between the MIA and saline injected

knees and for this study each measurement was the average of three separate measurements measured over 1 second. On day 15 the animals were euthanized and the right knees dissected out, disarticulated and the tibial plateaus photographed. The images were then divided into 4 regions, medial anterior and posterior, lateral anterior and posterior and each region scored for pathology by 5 blinded observers using a 0 to 6 scale. The total score for each joint was then calculated. Both the pain and pathology results were analyzed using a student's t-test to compare the results from the two experimental groups.

Results: Pain - On each day that pain was measured the difference in weight bearing between the MIA and saline injected knees was significantly less in the rats dosed with Anakinra compared to those dosed with vehicle ($p < 0.001$). This difference in weight bearing resulted in approximately a 50% reduction in the pain response in the MIA model but had no effect on the cartilage degradation seen in this model.

Gross Pathology: In contrast to the pain results Anakinra had no effect in reducing the cartilage degradation seen in the MIA model. The average joint score for both groups was 2.69.

Conclusions: The IL-1RA antagonist was able to significantly reduce the pain measured in the MIA model but had no effect on the cartilage degradation seen in this model.

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GLUCOSAMINE AND ITS N-ACETYL-PHENYLALANINE DERIVATIVE STIMULATE CARTILAGE REPAIR AND AFFECT GENE EXPRESSION IN RABBIT EXPERIMENTAL OSTEOARTHRITIS

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Purpose: The aim of this study was to evaluate the effects of Glucosamine (GlcN) and its N-acetyl phenylalanine derivative (NAPA), in rabbits with experimental Osteoarthritis (OA).

Methods: Experimental OA was induced by two intra-articular injections of vitamin A (Vit A) in rabbit knees. A severe OA without exposure of subchondral bone was obtained 35 days after the first injection. Then, rabbit OA left knees were intra-articularly injected with 2.5 mM GlcN or 2.5 mM NAPA, right knees were left untreated as OA control. Animals intra-articularly injected with normal saline solution were used as healthy control. Rabbits were sacrificed 70 days following first Vit A administration for histological assessment (May Grunwald-Giemsa, Hematoxylin-Eosin and Alcian Blue staining) and molecular analysis (Real Time-PCR) of the knee joints. Cathepsin B enzymatic activity was analyzed in rabbit primary chondrocytes.

Results: Histological sections obtained from articular cartilage showed a major cellularity, a better tissue organization and a major presence of matrix components in NAPA-treated samples and to a lesser extent in GlcN-treated samples compared to cartilage sections from OA knees.

Molecular analysis of mRNA expression levels of several genes coding for matrix components and remodeling enzymes in OA samples showed a lower expression compared to healthy tissues. Whereas GlcN and NAPA-treated samples showed a restored expression levels compared to OA cartilage, fairly close to values found in healthy animals.

Previously, we reported an inhibitory effect of GlcN and NAPA on Cathepsin B enzymatic activity *in vitro* assay. In order to analyze the effects of GlcN and NAPA on Cathepsin B *in vivo*, we isolated rabbit primary chondrocytes from healthy cartilage. First passage culture chondrocytes were left untreated, treated with TNF α , with TNF α plus GlcN and with TNF α plus NAPA.

Cathepsin B enzymatic activity resulted inhibited by NAPA and to a lesser extent by GlcN.

Conclusions: The double effect exerted by GlcN and NAPA, improved expression level of genes involved in cartilage homeostasis and inhibitory effect on Cathepsin B enzymatic activity, may explain the cartilage repair observed by histological analysis of OA joints treated with GlcN and NAPA.

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MENISCAL TRANSECTION IN GUINEA PIGS AS A MODEL FOR THE EARLY STAGES OF OSTEOARTHRITIS

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Purpose: Failure to develop therapies for osteoarthritis patients suggests that the degenerative changes in the joint are probably no longer sensitive to treatment. It is therefore important that early diagnosis of osteoarthritis becomes available when treatment can still halt or even reverse the joint destruction. Our goal was to develop a guinea pig model with only mild forms of osteoarthritis that could serve as a model for early human osteoarthritis to discover early osteoarthritis biomarkers and to test new treatments that are aimed at intervention early in the disease process.

Methods: Osteoarthritis was induced by bilateral transection of the medial meniscus in Dunkin-Hartley guinea pigs using a surgical procedure that was minimally invasive to avoid cartilage damage due to inflammation and/or intra-articular bleeding. At 4, 8, and 12 weeks after surgery the severity of osteoarthritis was macroscopically and histologically assessed and serum and urinary biomarkers were measured. The effect of daily treatment with 0.15 mg/kg Risedronate, 20 mg/kg Pioglitazone, 5 mg/kg Anakinra, and 0.5 mg/kg Galardin was evaluated in a 12-week study design.

Results: Four weeks after meniscal transection small lesions in the cartilage were macroscopically observed at the medial side of the joint, starting at the femoral head and the central part of the tibial plateau. Clear, but mild progression of osteoarthritis was detectable over time. However, 12 weeks after surgery the lesions were still restricted to the medial side of the joint and did not reach into the subchondral bone. Mild signs of osteoarthritis, but significantly less than in the meniscal transection group, were observed in the control and sham groups as well due to the susceptibility of the strain to the spontaneous development of osteoarthritis. Increased cartilage destruction in the meniscal transection group was also histologically observed.

Of the urinary biomarkers tested, only CTX-II was significantly increased in the meniscal transection group, but only at week 8. No increase in urinary HP/LP ratio (a measure for the relative turnover of cartilage over bone metabolism) nor in serum COMP levels was observed in the meniscal transection group within 12 weeks after surgery.

Strategies for treatment were chosen such that different processes in osteoarthritis development were targeted: bone destruction (Risedronate), inflammation (Pioglitazone and Anakinra), and cartilage destruction (Galardin). Unfortunately, none of these treatments showed beneficial effects on the macroscopic score in the meniscal transection model.

Conclusions: Mild joint destruction was observed in the first 12 weeks after bilateral meniscal transection. However, these early degenerative changes in the cartilage could not be detected by classic biomarkers. Probably, the cartilage destruction is still too mild to be measured in the systemic circulation. Further research into new biomarkers is needed to detect and monitor the early stages of osteoarthritis. Proteomics and metabolomics hold promise for the future to find such biomarkers. Previously, an uri-